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Meiotic Analysis in Some Species of *Ranunculus* (*Ranunculaceae*) from the Western Himalayas

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Cytological studies were carried out on a population basis for documenting the genetic diversity in seven species of the genus *Ranunculus* from geographically different areas of Himachal Pradesh and Kashmir in the Western Himalayas. The studied species are *R. arvensis* (n = 16), *R. diffusus* (n = 14, 16), *R. hirtellus* (n = 14, 16), *R. hyperboreus* (n = 14, 16), *R. laetus* (n = 14, 16), *R. muricatus* (n = 16) and *R. sceleratus* (n = 14, 16). Based on x = 7, 8, the species are at tetraploid levels. Of these n = 14 for *R. hyperboreus* and *R. sceleratus* are new records of an additional cytotype at world level and India level, whereas, n = 16 for *R. muricatus* at India level.

Key words: Himachal Pradesh, Kashmir, meiotic abnormalities, *Ranunculus*.

The genus *Ranunculus* is commonly known as the buttercups or crowfeet and contains 600 species on worldwide basis (Emadzade and Horandl 2008) including 33 species from India (Sharma et al. 1993). The species of the genus are distributed mostly in temperate regions and cold tropical mountains within the altitudinal range of 400–3000 m and are characterized as annual or perennial herbs with tuberous root stocks; prostrate to creeping stem; radial and cauline often dissected leaves; solitary yellow flowers and fruit a head or spike of achenes. Several species of the genus are known for their medicinal and ethnobotanical uses (Sharma et al. 1993, Prieto et al. 2003, Abbasi et al. 2010). The genus is known to exhibit well marked inter- and intraspecific chromosomal diversity, both at diploid and polyploid levels. With an aim to mark out the intraspecific genetic variants of *Ranunculus*, to be recommended for pharmaceutical testing, meiotic studies were carried out on populations from geographically different areas of the Western Himalayas.

Materials and Methods

For meiotic studies, flower buds were collected from different localities of selected areas of the Western Himalayas (Table 1). Smears of appropriate sized flower buds were made after fixing these in Carnoy's fixative, using standard acetocarmine technique. Pollen fertility was estimated by mounting mature pollen grains in glycero-acetocarmine (1:1) mixture. Well-filled pollen grains with stained nuclei were taken as apparently fertile, while shrivelled and unstained pollen grains were counted as sterile. Photomicrographs of pollen mother cells and pollen grains were made from freshly prepared slides using Nikon 80i Eclipse Digital Imaging System. Voucher specimens

Table 1. Information about locality, altitude, accession number, chromosome numbers, figure number and ploidy level seven species of *Ranunculus* from Western Himalayas

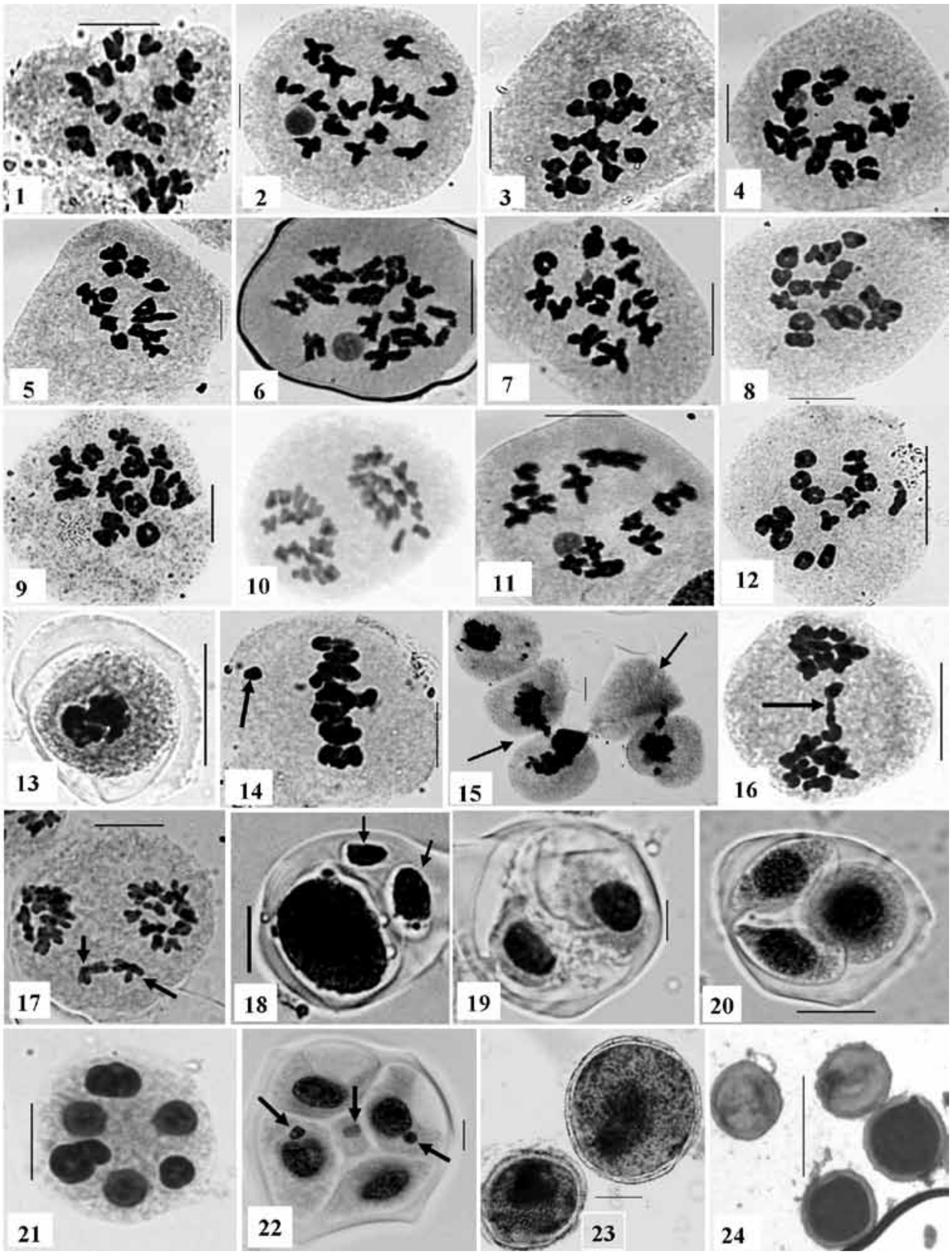
Taxa	Locality / Accession Altitude (m)	Accession numbers (PUN)	Meiotic chromosome number (n) / Figure number	Ploidy level
R. arvensis	Rehlu, District Kangra (H.P.); 890	52553	16 (Fig. 1)	4x
	Vikramvag, District Sirmaur (H.P.); 800	52666	16	4x
R. diffusus	Lohardhari, District Kangra (H.P.); 2300	52546	14 (Fig. 2)	4x
	Nauradhar, District Sirmaur (H.P.); 2100	52664	16 (Fig. 3)	4x
	Pahoo, District Pulwama (J & K); 1700	52466	16	4x
	Aharbal, District Kulgam (J & K); 2300	52465	16	4x
R. hirtellus	Dal- lake, District Kangra (H.P.); 2000	53598	14 (Fig. 4)	4x
	Yusmarg, District Budgam (J & K); 2400	53486	16 (Fig. 5)	4x
R. hyperboreus	Triund, District Kangra (H.P.); 3200	53597	14 (Fig. 6)	4x
	Haripur Dhar, District Sirmaur (H.P.); 3000	53596	16 (Fig. 7)	4x
	Chumnai, District Anantnag (J & K); 3500	53487	16	4x
R. laetus	Patti, District Kangra (H.P.); 900	52550	16 (Fig. 8)	4x
	Ranhear, District Kangra (H.P.); 800	52551	16	4x
	Ganduri, District Sirmaur (H.P.); 2600	52670	14 (Fig. 9)	4x
	Ratnipora, District Pulwama (J & K); 1750	52485	14	4x
R. muricatus	Patti, District Kangra (H.P.); 900	52548	16 (Fig. 10)	4x
	Laga, District Kangra (H.P.); 2000	52575	16	4x
	Boh, District Kangra (H.P); 1500	53599	16	4x
R. sceleratus	Shahpur, District Kangra (H.P.); 700	52552	14 (Fig. 11)	4x
	Patti, District Kangra (H.P.); 900	52554	16 (Fig. l2)	4x
	Sakiti, District Sirmaur (H.P.); 900	52665	16	4x
	Keller, District Shopion (J & K); 2200	52463	16	4x

H.P. = Himachal Pradesh, J & K = Jammu & Kashmir.

are deposited in the Herbarium, Department of Botany, Punjabi University, Patiala (PUN).

Observations

During the present study, 22 accessions belonging to seven species of the genus *Ranunculus* were cytologically investigated. The information about locality, altitude, present meiotic chromosome number, figure number and ploidy level are listed in Table 1. It is seen that chromosome number n = 16 is invariably counted in all the seven species. In five of these



Figs. 1–24. Chromosomes of seven *Ranunculus* species from the Western Himalayas.1. *R. arvensis* (n = 16), PMC at metaphase-I. 2. *R. diffusus* (n = 14), PMC at diakinesis. 3. *R. diffusus* (n = 16), PMC at metaphase-I. 4. *R. hirtellus* (n = 14), PMC at diakinesis. 5. *R. hirtellus* (n = 16), PMC at metaphase-I. 6. *R. hyperboreus*, (n = 14), PMC at diakinesis.7. *R. hyperboreus* (n = 16), PMC at diakinesis. 8. *R. laetus*, (n = 16), PMC at metaphase-I. 9. *R. laetus* (n = 14), PMC at metaphase-I. 10. *R. muricatus*16 chromosomes at each pole during A-I. 11. *R. muricatus* (n = 14), PMC at diakinesis. 12. *R. sceleratus,* (n = 16), PMC at metaphase-I. 13. PMCs showing chromosomal stickiness at metaphase-I. 14. PMC showing unoriented bivalent at metaphase-I. 15. PMCs involved in cytomixis showing Hypo- and Hyperploid cells. 16. PMC showing bridge at anaphase-I. 17. PMCs showing laggards at metaphase-II. 18. Monad with micronuclei. 19. Diad. 20. Triad. 21. Polyad. 22. Tetrad with micronuclei. 23. Heterogenous sized fertile pollen grains. 24. Fertile and sterile pollen grains. Scale: 10 µm.

Table 2. Data on cytomixis, meiotic course, pollen fertility and pollen grain size in different accessions of *Ranunculus* from the Western Himalayas

Taxa / Accession	Cytomixis		Meiotic course showing PMCs with				Pollen grains	
	Ratio of PMCs involved (%)	Number of PMCs involved	Chromosomal stickiness at M-I (%)	Unoriented bivalents at M-I (%)	Bridges at A-I &T-I / A-II & T-II (%)	Laggards at A-I & T-I / A-II & T-II (%)	Fertility (%)	Average Size (µm)
R. arvensis								
52553	3.42	2–3	6.83	2.34	6.42/4.43	4.00/4.64	92.80	31.72 × 29.47
52666	2.43	2–3	2.34	2.00	5.40/4.02	6.45/8.00	92.63	31.27 × 29.02
R. diffusus								
52546	22.48	2–4	4.40	3.64	7.80/7.80	3.00/3.50	78.04	27.02 × 26.44
52664	28.00	3–5	6.23	3.74	3.45/1.00	4.45/3.00	73.50	27.68 × 26.02
52465	26.30	2–4	5.60	3.23	7.04/4.30	8.00/8.10	79.60	26.96 × 25.05
52466	34.16	3–5	2.60	6.46	3.00/2.60	3.23/0	73.00	27.40 × 26.80
R. hirtellus								
53598	24.03	2–4	14.54	11.11	10.97/9.00	15.15/8.00	75.00	23.50 × 23.00
53486	23.63	3–5	10.11	11.45	11.00/7.10	10.45/9.00	76.00	23.80 × 22.48
R.hyperboreus								
53597	25.91	3–4	8.66	7.27	8.82/8.10	8.34/10.60	78.00	28.70 × 28.50
53596	28.00	2–5	29.47	0	10.54/6.98	10.00/10.00	76.00	28.87 × 27.50
53487	23.33	3–5	15.71	12.50	10.00/8.82	11.49/8.00	78.00	27.50 × 26.67
R. laetus								
52550	2.53	2–3	3.20	6.45	6.00/6.48	4.65/4.00	83.30	28.75 × 26.98
52551	3.20	2–3	4.32	10.72	10.00/7.00	7.40/4.06	92.00	28.14 × 27.14
52670	1.25	2–3	4.45	13.34	4.45/0	3.20/3.28	93.00	27.90 × 27.10
52485	6.78	2–3	0	0	11.50/0	3.04/4.00	87.00	27.77 × 26.93
R. muricatus								
52548	3.45	2–3	0	0	2.00/2.52	10.05/2.00	86.50	29.50 × 32.43
52575	4.07	2–3	0	0	2.78/2.00	12.58/0	93.48	30.02 × 28.41
53599	2.30	2–3	8.75	10.00	5.75/1.00	12.80/0	88.00	28.90 × 31.20
R. sceleratus								
52552	3.42	2–3	12.40	8.48	6.00/4.50	5.58/5.00	90.00	28.24 × 25.09
52554	4.50	2–3	2.23	4.82	6.75/2.00	5.43/0	93.68	29.05 × 25.00
52665	7.52	2–3	4.20	2.41	4.10/0	7.23/0	89.00	27.72 × 26.50
52463	2.32	2–3	1.49	2.13	12.75/6.00	5.84/6.84	89.00	28.45 × 26.32

Table 3. Data on abnormal microsporogenesis in different accessions of *Ranunculus* from Western Himalayas

Taxa / Accessions	Monads		Diads		Microsporogenesis Triads		Tetrads		Polyads	
	WMN*	WM**	WMN	WM	WMN	WM	WMN	WM	WMN	WM
R. arvensis										
52553 (131)	9.17	11.46	9.91	8.40	8.40	0	29.78	14.50	4.58	3.81
52666 (155)	9.04	4.52	6.46	5.16	11.61	6.45	31.62	18.06	4.50	2.58
R.diffusus										
52546 (110)	10.00	3.64	8.18	6.37	10.90	8.19	34.54	18.18	0	0
52664 (131)	6.87	3.81	10.68	5.34	6.10	6.87	41.22	12.21	3.81	3.08
52465 (130)	10.76	7.69	8.46	0	9.24	0	44.62	16.16	3.07	0
52466 (142)	9.85	2.81	3.52	3.52	8.45	0	44.36	19.71	4.92	0
R. hirtellus										
53598 (122)	0	3.27	17.21	2.45	15.57	1.63	31.15	22.96	4.09	1.63
53486 (178)	5.05	6.74	1.68	5.05	1.68	1.66	53.93	23.03	0.56	0.56
R.hyperboreus										
53597 (178)	0	0	2.24	1.13	4.49	1.13	85.39	5.62	0	0
53596 (141)	8.51	4.25	4.96	2.83	3.54	1.41	48.22	22.69	2.83	0.70
53487 (118)	0	5.08	0	1.69	3.38	1.69	61.01	25.42	0.84	0.84
R. laetus										
52550 (118)	7.83	0	9.38	5.46	14.06	5.46	32.05	23.43	1.56	0.78
52551 (100)	5.00	0	17.00	2.00	15.00	0	32.00	25.00	4.00	0
52670 (101)	0.99	0.99	0	0	3.48	0.99	83.58	9.97	0	0
52485(133)	2.28	1.50	11.15	4.51	15.78	4.51	33.38	24.06	0	2.22
R. muricatus										
52548 (159)	0	0	7.54	2.55	11.32	3.77	47.16	23.89	3.77	0
52575 (170)	2.94	0	5.88	2.94	8.82	2.94	49.41	24.70	1.17	1.17
53599 (105)	6.67	10.74	0	0	6.67	0	66.67	9.52	0	0
R. sceleratus										
52552 (161)	0	0	3.10	1.86	6.21	0	61.49	24.84	3.10	0
52554 (162)	4.32	1.23	5.55	3.70	3.70	3.70	56.79	19.75	1.23	0.62
52665 (120)	4.16	0	9.16	1.66	14.65	5.00	46.66	17.50	0.81	0
52463 (123)	8.13	0	9.75	0	5.69	0	51.21	25.57	0	0

*WMN = without micronuclei (%); **WM = with micronuclei (%).
Figures in parentheses denote and total number of PMCs observed.

species, n = 14 is also exhibited in some of the accessions. Further, it is seen that in species with different populations exhibiting either n = 14 or n = 16, the latter number is found to be more common. The bivalents are quite large with 1–2 chiasmata observed in almost all the species.

Meiotic abnormalities

The meiotic course in all these accessions has been observed to be abnormal with the presence of cytomixis, chromosome stickiness, unoriented bivalents, chromatin bridges, laggards at anaphases and telophases, formation of micronuclei and abnormal microsporogenesis leading to variable pollen sterility (Tables 2, 3).The cytomixis remained the chief phenomenon of the meiotic system in all the 22 accessions. The cytomixis in the form of cytoplasmic connections/ channels as well as transfer of chromatin between PMCs is seen right from prophase-I to pollen formation and may involve 2–5 PMCs (Fig. 15). Cytomixis in these species results into the production of PMCs with different chromosome numbers and even empty PMCs (Fig. 15). The frequency of cytomixis has been observed to be variable with the highest being noted in the accessions of *R. diffusus* (Table 2). It is seen that mostly late or non-disjuncting bivalent bridges as well as chromosomal laggards are more common

(Figs. 16, 17). Chromatin stickiness involving few bivalents or whole complement is seen from prophase-I to metaphase-I (Fig. 13). The unoriented bivalents at metaphase-I (Fig. 14) have also been observed with highest frequency noted in the accessions of *R. laetus* (Table 2). The cytomixis inducing abnormalities ultimately end up in multipolarity with the formation of highly variable number of nuclei per PMC. This results in abnormal microsporogenesis leading to the formation of monads, diads, triads and polyads (Figs. 18–21). Further, micronuclei have also been observed in most of these species (Table 3, Figs. 18, 22). All these meiotic abnormalities lead to the formation of heterogeneous sized fertile pollen grains in all the accessions (Fig. 23). It has also been observed that in the accessions with low frequency of cytomixis, the pollen grain fertility is not seriously affected as compared to the accessions of *R. diffusus*, *R. hirtellus* and *R. hyperboreus*, in which the frequency of cytomixis is very high and thus reducing the pollen grain fertility (Fig. 24, Table 2).

Discussion

A perusal of cytological cumulative literature brings to light that 320 species/461 cytotypes of the genus *Ranunculus,* including 20 species from India, have been cytologically worked out. The chromosome numbers in the genus vary considerably (2n = 14 to 144) with 2n = 16 (41.21%) being the most common followed by 2n = 32 (25.59%), 2n = 48 (11.71), 2n = 14 (4.77%), 2n = 24 (4.33%), 2n = 28 (4.12%), 2n = 40 (3.03%), 2n = 96 (1.51%), 2n = 64 (1.30%) while 2n = 26, 33, 34, 42, 56, 128, 144 are relatively less common on a worldwide basis (c.f. Fedorov 1969, Kumar and Subramanian 1986, Index to Plant Chromosome Numbers, web, etc.). However, from India of the different chromosome numbers (2n = 16, 24, 28, 32, 40, 42, 46, 48, 56) one with 2n = 32 is the most common. Thus the genus is polybasic (x = 6, 7, 8). Being most prevalent and the occurrence of polyploidy series on n = 8, x = 8 is the original base number and x = 7 being derived through aneuploid reduction. Further, the prevalence of aneuploid cytotypes is also very clear. However, the base number x = 6 is only speculative in a way to relate it to 2n = 24 chromosome reports to be taken as tetraploids at par with 2n = 28, 32. Polyploidy in the genus *Ranunculus* is common. The highest level of polyploidy is deciphered as approximately 20x, in *R. glabrifolius* with 2n =144 (Rendle and Murray 1989), otherwise the frequency of the tetraploids is the highest. Polyploid levels range from 4x–20x on a worldwide basis but in India it is limited to 8x level. The chromosome count of n = 14 in *R. hyperboreus* Rottb., is new for the species on a worldwide basis, as the species is already known to have 2n = 24 (Sokolovskaya 1968), 2n = 32 (Zhukova 1982) and 2n = 64 (Mosquin and Hayley 1966). Other reports given here are in conformity with the previous reports from different parts of the world. Most of these studied species are from unexplored areas of India and are made on a population basis. Thus polyploidy and aneuploidy remains the chief modes of evolution and speciation in the genus *Ranunculus.*

Cytomixis, co-existing with meiotic abnormalities in all these materials further give a clue that cytomixis definitely causes disturbance in the formation and functioning of the spindle during meiosis, resulting in irregular distribution of the chromatin within the PMCs and also between the PMCs showing the transfer of chromatin. Although in low frequency, the occurrence of PMCs with high and low chromosome numbers is observed, leading ultimately to the induction of abnormal polarity of spindle and formation of nuclei of variable numbers and sizes during microsporogenesis and finally resulting in the production of heterogenous sized fertile pollen grains (Table 2). All these findings are in conformity with earlier observations in different plants (Haroun et al. 2004, Ghaffari 2006, Singhal et al.

2008). Cytomixis leading to the production of aneuploids (Sheidai and Fadael 2005) and also of higher ploidy levels (Semyarkhina and Kuptsou 1974) seems to be equally operative in the presently investigated species. It is noted that cytomixis and meiotic abnormalities make integral appearance in all the accessions but in varying frequencies (Table 2). Hence, in general, the pollen fertility is not drastically affected in species with low frequency of cytomixis. Similar observations concerning the low frequency of cytomixis coupled with limited appearance of meiotic abnormalities and negligible low pollen sterility has also been reported in *Dactylis* (Falistocco et al. 1995) and *Hippophae rhamnoides* (Singhal et al. 2008). The micronuclei observed in many PMCs may be due to the extra chromatin mass transferred by cytomixis as has also been suggested by Bhat et al. (2006). This phenomenon of cytomixis has been seen in many of the angiospermic species with different possible causes being advocated as pathological/physiological conditions, the effect of chemicals, temperature, stress factors or purely under genetic control. Since all the accessions have been meiotically processed following the same standardized protocol of fixation, cytomixis in *Ranunculus* seems to be under genetic control as pointed out earlier by some researchers in other taxa (Bellucci et al. 2003, Fadaie et al. 2010).

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S. Kumar，S. M. Jeelani，S. Rani，R. C. Gupta，S. Kumari：**西ヒマラヤ産キンポウゲ属（キンポウゲ科）数種に関する減数分裂の解析**

西ヒマラヤ・ヒマチャルプラデシュとカシミール産のキンポウゲ属（キンポウゲ科）7 種の細胞学的な解析を集団レベルで行った．得られた結果は次のとおりであった；*Ranunculus arvensis* (n = 16), *R. diffusus* (n = 14, 16), *R. hirtellus* (n = 14, 16), *R. hyperboreus* (n = 14, 16), *R. laetus* (n = 14, 16), *R. muricatus* (n = 16), *R. sceleratus* (n = 14, 16)．基本数 x = 7, 8 にもとづくと，これらの植物は四倍体であった．これらの算定のうち，*R. hyperboreus* と *R. sceleratus* の n = 14 は新たなサイトタイプであり，*R. muricatus* の n = 16 はインドでは初めての報告である．

（インド・Punjabi 大学）